

## CLAIMS

1°) Substantially pure mammal polypeptide, characterized in that it contains sites such that when said sites are exposed at the surface of a cell, they are able of binding iodocyanopindolol (ICYP) under blockade of  $\alpha$ ,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ -AR, serotonin 5-HT<sub>1A</sub> and serotonin 5-HT<sub>1B</sub> receptors, said binding being saturable, reversible, able to be displaced by a  $\beta$ -adrenergic receptor agonist SM-11044 with stereoselectivity but not by isoproterenol, norepinephrine, epinephrine, serotonin, dopamine or BRL-37344, and not being blocked by propranolol, said polypeptide (1) having an apparent molecular weight of about 30-40 kDa when labeled with <sup>125</sup>I-iodocyanopindolol after photoaffinity labeling and separation by electrophoresis and an apparent molecular weight of about 60-80 kDa in Western blot, and (2) generating a fragment having the following formula DPX<sub>1</sub>FFQHRIHX<sub>2</sub>FSIFNX<sub>3</sub> by acidic cleavage, wherein, X<sub>1</sub> represents S (SEQ ID N°5) or X (SEQ ID N°6), X<sub>2</sub> represents V (SEQ ID N°6) or W (SEQ ID N°5) and X<sub>3</sub> represents S (SEQ ID N°5) or H (SEQ ID N°6), said polypeptide being present at least on muscles and eosinophils membranes and being a non-adrenergic receptor.

2°) The polypeptide according to claim 1, characterized in that it contains at least SEQ ID NO:1.

3°) The polypeptide according to claim 1, characterized in that it consists of SEQ ID NO:13.

20 4°) An isolated and purified nucleic acid sequence, characterized in that  
it encodes a mammalian receptor as claimed in claim 1.

5°) The isolated and purified nucleic acid sequence of claim 4, characterized in that it includes at least SEQ ID NO:2.

6°) The isolated and purified nucleic acid sequence of claim 4,  
25 characterized in that it consists of SEQ ID NO:14.

7°) The purified nucleic acid sequence according to claim 4 or claim 5, characterized in that it hybridizes with SEQ ID NO:3 or SEQ ID NO:4.

8°) cDNA clones, characterized in that they comprise a sequence coding for the instant non-adrenergic receptor according to claim 1.

[illegible]

9°) Synthetic or non-synthetic nucleotide probes, characterized in that they hybridize with a nucleic acid according to claims 4 to 8 or with their complementary sequences or their corresponding RNA, these probes being such that they do not hybridize with the genes or the messenger RNA coding for  $\beta$ -adrenergic receptors.

5 10°) Probes according to claim 9, characterized in that they are selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:7 to SEQ ID NO:12, optionally labeled using a label such as a radioactive isotope, a suitable enzyme or a fluorochrome.

10 11°) Primers for amplifying a nucleic acid sequence according to claims 4 to 8, characterized in that they are selected from the group consisting of SEQ ID NO:7 to SEQ ID NO:12.

12°) A recombinant plasmid in particular for cloning and/or expression, containing a nucleic acid sequence according to claims 4 to 8 at one of its cloning sites non essential for its replication.

15 13°) The recombinant plasmid according to claim 12, characterized in that it further comprises an origin of replication for replication in a host cell, at least one gene whose expression permits selection of said host cell transformed with said plasmid, and a regulatory sequence, including a promoter permitting expression of a polypeptide having a non-adrenergic activity as defined hereabove, in said host cell.

20 14°) The recombinant plasmid according to claim 12, characterized in that said plasmid is pcDNA3 into which is inserted, in a multisite linker, SEQ ID NO:2, wherein said plasmid is deposited with the Collection Nationale de Cultures de Microorganismes [National Collection of Microorganism Cultures] (CNCM held by the PASTEUR INSTITUTE, dated December 10, 1996, under No. I-1795.

25 15°) A host cell transformed by a recombinant plasmid according to claims 12 to 14, characterized in that it comprises the elements of regulation making possible the expression of the nucleotide sequence coding for the instant polypeptide in this host.

0931974-090899

16°) The host cell according to claim 15, characterized in that it consists, in particular, in mammalian cell lines.

17°) Antibodies directed specifically against the instant polypeptide according to claim 1, these antibodies being such that they recognize neither known  $\alpha$  or  $\beta$ -adrenergic, nor serotonin, nor dopamine receptors.

18°) A method for assaying a substance for agonist or antagonist activity towards a polypeptide according to claim 1, which method comprises:

- placing the substance in contact with tissue membrane proteins or a transformed host cell expressing a polypeptide according to claim 1 under conditions which permit binding between said polypeptide binding sites and an agonist or an antagonist thereto and

- measuring an appropriate transduction signal.

19°) A process for studying the binding affinity of a compound for a polypeptide according to claim 1, which process comprises:

- transforming a host cell by an expression vector comprising a nucleotide sequence coding for the instant receptor,

- culturing said transformed host cell under conditions which permit the expression of said receptor encoded by said nucleotide sequence and the transfer of the expressed receptor polypeptide to the membrane of the said transformed host cell so that transmembrane sequences of said receptor polypeptide are embedded in the cell membranes of the transformed host cell;

- placing said transformed host cell in contact with said compound and  
- measuring the quantity of said compound bound to said receptor polypeptide.

20°) A process for studying the binding affinity of a compound for a polypeptide according to claim 1, which process comprises:

- extracting membrane proteins corresponding to the instant receptor polypeptide from appropriate tissues or cells such as muscles,

- placing said membrane proteins in contact with said compound and

0531974-090899

- measuring the quantity of said compound bound to said receptor polypeptide.

21°) Method of preparation of a polypeptide according to claim 1, which method comprises:

5 ----- - preparing membrane proteins from a tissue containing the instant polypeptide, such as rat colon or human skeletal muscle,

- labeling said membrane proteins with [ $^{125}$ I]-ICYP-diazirine or another appropriate marker under blockade of  $\alpha$ ,  $\beta$ 1,  $\beta$ 2,  $\beta$ 3-AR and serotonin receptors,

10 phoresis and  
- separating said labeled proteins by preparative SDS-PAGE electro-

- extracting the radioactive band.

add Q 17  
add  
\$10

668060-12267E60